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Basu, Kaninika; Inglis, Sarah K.; Bremner, Stephen A.; Ramsay, Rebecca; Abd, Ali; Rabe, Heike

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Filaggrin gene defects are associated with eczema, wheeze and nasal disease during infancy: prospective study

Kaninika Basu, MD^{1,2}, Sarah K Inglis, PhD³, Stephen A Bremner, PhD⁴ Rebecca Ramsay, DHECN⁵, Ali Abd, MBChB, MSc², Heike Rabe, MD PhD^{2,5}, Elizabeth Strange, BMBS², Veronica Phillips, PhD⁶, Paul Seddon, FRCPCH^{2,5}, Roger Tavendale, PhD⁷, Anjum Memon, DPhil⁴, Colin N A Palmer, PhD⁷, Katy Fidler, PhD^{2,5}, Somnath Mukhopadhyay, PhD^{2,5}

¹ Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK; ² Academic Department of Paediatrics, Royal Alexandra Children's Hospital, Brighton and Sussex Medical School, Brighton, UK; ³Tayside Clinical Trials Unit, University of Dundee, UK; ⁴Department of Primary Care and Public Health, Brighton and Sussex Medical School, Brighton, UK; ⁵Brighton and Sussex University Hospitals NHS Trust, Brighton, UK; ⁶Medical Library, University of Cambridge, Cambridge, UK; ⁷Biomedical Research Institute, Ninewells Hospital and Medical School, University of Dundee, UK.

Corresponding author:

Dr Kaninika Basu, MD

Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK

Email: basukaninika@gmail.com

Telephone: +44 7810 828459

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30 **Capsule Summary**

31 This prospective cohort study describes associations between the presence of filaggrin gene
32 mutations and eczema, rhinitis and wheeze from as early as age six months, raising new
33 questions regarding underlying mechanisms and timing of interventions.

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Filaggrin gene defects are associated with eczema, wheeze and nasal disease during infancy: prospective study

To the Editor:

The protein filaggrin (FLG) is present in the skin and nasal epithelium and helps maintain the skin barrier while performing other functional roles¹⁻³. Many studies have related the presence of common, loss-of-function filaggrin gene (*FLG*) defects to the incidence and severity of eczema³ and the severity of asthma^{4,5} during childhood.

As allergy-related diseases, such as eczema or wheeze, often start from early infancy, it is important to explore whether the presence of *FLG* gene defects influences symptoms during infancy (6 and 12-month time points) and how these relate to progression of symptoms beyond infancy. If filaggrin does influence allergy-related symptom status during infancy, there may be justification for trialling interventions starting soon after birth, targeted towards infants with adverse *FLG* genotype, in order to explore beneficial effects on eczema, wheeze and other clinical outcomes as early as 6 and 12 months. It is known that *FLG* defects are associated with impaired skin barrier function, while skin barrier function defects are potentially correctable through the use of regular treatments⁶.

A systematic review (December 2018) identified only two studies^{7,8}, both retrospective, that have explored the link between filaggrin gene defects and allergy-related symptoms below 12 months. Repeated measurements analyses in the Isle of Wight cohort showed that *FLG* defects were associated with an almost 3-fold increased risk of eczema during the first 12 months of life. Nine out of nine infants with eczema at 3 months continued to have eczema at 6 months of age⁶. This supported our retrospective analyses showing that presence of *FLG* mutations in two cohorts of modest size (Copenhagen n=379; Manchester n=503) was associated with a significant increase in eczema risk before the age of 12 months⁷. There was a significant enhancement of this risk with cat ownership at birth, thus adding further strength to the hypothesis that the genetically driven skin barrier defect may be playing a causal role through allergen entry⁷. We designed a prospective study to define the role of *FLG* gene defects on allergy-related outcomes during infancy.

2312 pregnant women were recruited to the GO-CHILD study between 2009 and 2015 from 8 National Health Service (NHS) Trusts in England and Scotland. The study was approved by the Tayside Committee on Medical Research and Ethics. Expectant mothers were invited during antenatal visits and a cord blood sample at birth or saliva in the postnatal period was

collected for genotyping. (See Online Methods for details) The cord blood samples were stored at -80°C. Cord blood and saliva samples were transported to the University of Dundee for genotyping. Infants with severe perinatal problems or congenital anomalies were excluded from the subsequent follow-up. The children were followed up for symptoms related to atopy at the ages of 6, 12 and 24 months by postal questionnaires sent to the carers (online Methods). Online Figure 1 shows the methodology and Online Table 1 describes the demographic characteristics of the cohort. Questions related to dry skin, eczema, wheeze, upper respiratory conditions and food allergies, and how these symptoms affected the child's life, including any visits to primary or secondary care and the prescribing of medication. For simplicity and greater accuracy through recall, responses for any of the three options - yes, no, don't know - were used for analysis. 'Wheeze' was defined as 'breathing that makes a high-pitched whistling or squeaking sound from the chest, not the throat' and 'rhinitis' was defined as "a problem with sneezing, or a runny, or blocked nose when he/she did not have a cold or the flu".

All genetic analyses were anonymised. Genotyping for *FLG* R501X, 2282del4, S3247X and R2447X was performed as described in earlier papers³⁻⁵. AA refers to the wild-type *FLG* genotype, Aa refers to heterozygous genotype with one of the mutations, and aa refers to homozygous genotype. The homozygous, heterozygous and compound heterozygous genotypes were considered together as Aa/aa. Data analyses were conducted using the IBM SPSS Statistics 146 software, Version 23 (IBM Corp., Armonk, and New York, USA), Stata version 15.2(College Station, TX: StataCorp LLC) and Instat for Macintosh programmes. Binary logistic regression was used to estimate e odds ratios for dry or itchy skin for comparing the effects of the mutations. For atopic outcomes i.e. eczema, wheeze and rhinitis, log-binomial regression was used to estimate relative risks.. Attendance at day-care and exposure to animals were included in all models as covariates after stepwise removal procedures (covariates with $p < 0.05$ were retained). Exposure to smoke, another potential covariate, did not contribute significantly to the model and was not associated with genotype in any subgroup tested and hence was excluded from the final analysis.

The presence of dry or itchy skin, and a parent-reported diagnosis of eczema were found to be significantly increased in children with any *FLG* mutation at all the three time-points (6 months, 1 year, 2 years following birth). At 6 months of age, the heterozygous and homozygous genotypes for any of the *FLG* mutations were associated with higher risk of

eczema (RR 1.82, 95%CI 1.39-2.39), dry or itchy skin (OR 2.71, 95%CI 1.61-4.55), wheeze (RR 1.63, 95%CI 1.00-2.65) and rhinitis (RR 1.46, 95%CI 1.06-2.01), compared to the wild type (Online Table 2). At age 1 year, the presence of one or more *FLG* mutations continued to be associated with higher risk of increased eczema (RR 1.80, 95%CI 1.39-2.32) and odds of dry or itchy skin (OR 2.28, 95%CI 1.32-3.92) compared to wild type, however, the association with wheeze and rhinitis were not significant (Online Table3). At age 2 years, the presence of one or more *FLG* mutations was associated with significantly higher risk of eczema (RR 1.40, 95%CI 1.00-1.97) and odds of dry or itchy skin (OR 1.83, 95%CI 1.02-3.28), The associations with wheeze and rhinitis were not significant (Online Table 4). We report the results of the repeated measurements analysis in Table 5.

Three-hundred thirty-one families returned completed questionnaires for their children at all three time-points, 6 months, 1 year and 2 years. This allowed us to explore whether there are differences in the time-course of allergy-related events in filaggrin-sufficient versus filaggrin-deficient infants and children over the first two years of life. Infants and young children with *FLG*-deficient status were more likely to suffer from eczema; estimated difference in proportions (d) 0.12(95% CI, 0.01 to 0.24) and rhinitis; $d=0.10$ (95% CI, 0.02 to 0.19) over 6-24 months in comparison to those with *FLG*-sufficient status; however, there were no observed differences for wheeze; $d=0.04$ (95% CI, -0.05 to 0.14). The bar charts show that, for those with a filaggrin mutation, prevalence (red portion of bar) of wheeze, eczema and rhinitis are all greater in the past 6, 12 and 24 months compared to those without a mutation. This pattern is less pronounced for wheeze and no difference was observed at 24 months. (online Figure 2)

This is the first prospective study exploring the role of *FLG* gene defects on allergy-related disease outcomes at age 6 months. It indicates that the presence of one or more *FLG* gene defects from birth influences multiple aspects of allergy-related disease, including eczema, wheeze and nasal disease, at early infancy. The increased risk of filaggrin-associated nasal symptoms in 6-month olds may involve interactions between filaggrin deficiency states and allergen exposures to the nose occurring very early in life. The presence of *FLG* defects may

also define a sub-phenotype of allergy-related disease that manifests over the first 2 years of life, with implications for allergy-related disease phenotype over later childhood. There are some limitations of this study. We did not plan and perform a formal *a priori* calculation of sample size and this is a weakness of our study. However, the rationale underlying our choice of sample size⁹ is presented in Online Methods. The sample size for all the analyses reported in this paper is at least twice the figure of 150 recommended by the paper⁹. We thus feel there is a high expectation that these results are valid and can be replicated in future meta-analyses⁹. In addition, the recommendations⁹ refer to case-control studies, whereas this is a longitudinal cohort study, which has a more robust design. Findings of association studies must be supported by independent replication, with associations combining family- based and population- based analysis, with an odds ratio/relative risk and/or attributable risk that is high⁹. However, we have not found any published data to compare to and thus replicate our findings. Being a prospective birth cohort study, there is a fair amount of work for new mothers in terms of completing the questionnaires and hence there was a relatively low return rate and complete data comprising all three questionnaires. We are also unable to comment on any possible effect of ethnicity variations as the majority of the participants were Caucasian. Future interventional studies directed at *FLG*-deficient populations from birth may show improvements in clinical outcomes beyond eczema from as early as 6 months of age.

K Basu, MD^{1,2}

S K Inglis, PhD³

S A Bremner, PhD⁴

R Ramsa, DHECNy⁵

A Abd, MB.ChB, MSc²

H Rabe MD PhD^{2,5}

E Strange, BMBS²

V Phillips, PhD⁶

P Seddon, FRCPCH^{2,5}

R Tavendale, PhD⁷

159 *A Memon, DPhil⁴*

160 *C N A Palmer, PhD⁷*

161 *K Fidler, PhD^{2,5}*

162 *S Mukhopadhyay, PhD^{2,5}*

163

164 *¹ Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK*

165 *² Academic Department of Paediatrics, Royal Alexandra Children's Hospital, Brighton and*

166 *Sussex Medical School, Brighton, UK*

167 *³ Tayside Clinical Trials Unit, University of Dundee, UK*

168 *⁴ Department of Primary Care and Public Health, Brighton and Sussex Medical School,*

169 *Brighton, UK*

170 *⁵ Brighton and Sussex University Hospitals NHS Trust, Brighton, UK*

171 *⁶ Medical Library, University of Cambridge, Cambridge, UK*

172 *⁶ Biomedical Research Institute, Ninewells Hospital and Medical School, University of*

173 *Dundee, UK.*

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Figure Legends

Figure 1: Study design

Figure 2: Incidence (%) of atopy-related clinical outcomes during the first 2 years of life in children where 6-month, 12-month and 24-month follow-up data are available (n=331)

Any filaggrin mutation No/Yes: history of eczema

KEY:

Blue bar: No

Red bar: Yes

Any filaggrin mutation No/Yes: history of dry of itchy skin

KEY:

Blue bar: No

216 Red bar: Yes
217
218 Any filaggrin mutation No/Yes: history of wheeze
219 *KEY:*
220 Blue bar: No
221 Red bar: Yes
222
223 Any filaggrin mutation No/Yes: history of rhinitis
224 *KEY:*
225 Blue bar: No
226 Red bar: Yes
227
228

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The Influence of genetic and environmental factors on childhood diseases

Postal questionnaire to assess asthma/allergy and infection

Thank you for agreeing to participate in our study. Please fill out this questionnaire with the information about the first 6 months of your child's life and return this in the pre-paid envelope that has been supplied.

Name of Child:

Bar Code Sticker

CHI / NHS Number:

Date of Birth:

- Person completing questionnaire (tick box please):

Mother ☐ Father ☐ Other ☐

- Date questionnaire completed:

day ____ month ____ year ____

(please fill in today's date)

Bar code sticker

Questions on wheezing

By “wheezing” we mean breathing that makes a high-pitched whistling or squeaking sound from the chest, not the throat

1. Has your child had wheezing or whistling in the chest in the last 6 months? Yes/ No

If you answered “no” please skip to question 11.

2. How old was your child when he/she first began to wheeze?

_____ years _____ months

3. In the first 6 months, has your child had wheezing or whistling in the chest during or soon after a cold or flu? Yes/ No

4. In the first 6 months, has your child had wheezing or whistling in the chest even without having a cold or flu? Yes/ No

5. How many attacks of wheezing has your child had during the first 6 months?

☐ None ☐ 1 to 3 ☐ 4 to 12 ☐ more than 12

6. Do these attacks cause him/her to be short of breath?

☐ yes, always ☐ most of the time ☐ occasionally ☐ no, never

7. Which of these two descriptions fits best your child’s wheeze? (tick one only)

a) My child has only short attacks of wheeze, for example with colds. In between these attacks, he/she does not normally wheeze. ☐

b) My child wheezes always or a lot of the time. With colds he/she has attacks with more severe wheeze. ☐

8. In the first 6 months, how often, on average, has your child’s sleep been disturbed due to wheezing?

☐ never woken with wheezing ☐ less than one night per week
☐ one or more nights per week

9. In the first 6 months, how much did wheezing interfere with your child’s daily activities?

☐ not at all ☐ a little ☐ a moderate amount ☐ a lot

10. In the first 6 months did the following things cause wheezing in your child?

- exercise (playing) ☐ yes ☐ no ☐ don't know
- laughing, crying or excitement ☐ yes ☐ no ☐ don't know
- contact with pets or other animals ☐ yes ☐ no ☐ don't know
- food or drinks ☐ yes ☐ no ☐ don't know

11. In the first 6 months, did your child suffer from rattly breathing (rattles)?

- ☐ never ☐ only with a cold
- ☐ sometimes even without a cold ☐ almost always

12. Does your child attend day care or nursery? Yes/ No

13. Was your child breastfed? Yes/ No

If yes, how long: ☐ less than a month ☐ 1-3 months ☐ 4-6 months

14. During the first 6 months of life, did your child posit or vomit?

- ☐ not at all ☐ a little ☐ a lot

15. Has your child had an itchy rash at any time in the first 6 months? Yes/ No

Has your child had this itchy skin condition in the last week? Yes/ No

How old was your child when this condition began.....

Has this skin condition ever affected the skin creases in the past – by skin creases we mean fronts of elbows, behind the knees, front of ankles, around the neck or around the eyes?

Yes/ No

16. In the first 6 months, has your child suffered from a dry skin in general? Yes/ No

Has your child suffered from any of the following skin complaints (Please tick one or more)

- ☐ Eczema ☐ Cradle cap ☐ Nappy rash ☐ Facial spots ☐ Heat rash

17. If your child had a rash did you think at the time that this was related to your washing powder?

Yes/ No

What type of washing powder were you using at the time of the rash?

- ☐ Biological ☐ Non-biological ☐ Not sure

Questions on ears, nose and throat

18. In the first 6 months, how many times has your child had a cold or flu?

- ☐Never ☐1 - 3 times ☐4 - 6 times
☐7 -10 times ☐more than 10 times

19. How long does a cold usually last in your child?

- ☐less than 1 week ☐1 to 2 weeks
☐2 to 4 weeks ☐more than 4 weeks

20. In the first 6 months, has your child had a problem with sneezing, or a runny, or blocked nose when he/she did NOT have a cold or the flu? Yes / No

21. In the first 6 months, how much did this nose problem interfere with your child's daily activities?

- ☐Not at all ☐a little ☐a moderate amount ☐a lot

22. Over the first 6 months, has your child snored or had a blocked nose at night?

Yes/ No

If yes, how often: ☐only with a cold ☐sometimes even without a cold
 ☐almost always

23. Did the snoring/blocked nose disturb your child's sleep?

- ☐not at all ☐a little ☐a moderate amount ☐a lot

Questions on coughing

24. Does your child usually have a cough with colds? Yes/ No

25. Does your child have a cough even without having a cold?

- ☐No, never ☐yes, sometimes ☐yes, always

26. Do you think that your child coughs more than other children? Yes/ No

27. In the first 6 months, has your child had a dry cough at night, apart from a cough associated with a cold or a chest infection? Yes/ No

28. In the first 6months, did the following things cause coughing in your child?

- | | | | |
|--------------------------------------|------------------------------|-----------------------------|-------------------------------------|
| • exercise (playing) | <input type="checkbox"/> yes | <input type="checkbox"/> no | <input type="checkbox"/> don't know |
| • laughing, crying or excitement | <input type="checkbox"/> yes | <input type="checkbox"/> no | <input type="checkbox"/> don't know |
| • contact with pets or other animals | <input type="checkbox"/> yes | <input type="checkbox"/> no | <input type="checkbox"/> don't know |
| • food or drinks | <input type="checkbox"/> yes | <input type="checkbox"/> no | <input type="checkbox"/> don't know |

Questions on your household

29. Do you keep any household pets? Yes/No
 If yes, do you keep any of these pets? (tick as many as apply)
☐Dog ☐Cat ☐Other furry pets ☐Bird
30. Is the child exposed to smoking? Yes/No
31. Does the child's mother smoke cigarettes? Yes/No
 If yes, how many per day? ☐1 to 10 ☐11 to 20 ☐more than 20
32. Do any other household members smoke cigarettes? Yes/No
 If yes, how many per day (total of cigarettes)?
☐1 to 10 ☐11 to 20 ☐more than 20
33. How would you describe the location of your house?
☐ In a street with very dense traffic (main road)
☐ In a street with moderate traffic (residential road)
☐ In a quiet street with little or no traffic

Questions about Infection

Hospital Admissions

Was your baby admitted to the Special Care Baby Unit? Yes/No

- If YES why?

☐ Premature

☐ Breathing problems

☐ Suspected infection

☐ Confirmed infection

- If infection what kind? ☐ Chest ☐ Meningitis ☐ Blood ☐ Other?

.....

- Has your child had any/other admissions to hospital? Yes/No

If YES, How many times?

Please fill this table for further information about your child hospital admission

Name of hospital and Date of admission	Age	Diagnosis	Treatment
1 st Admission Name of hospital Date of Admission:		Meningitis <input type="checkbox"/> Pneumonia <input type="checkbox"/> Bronchiolitis <input type="checkbox"/> Wheeze <input type="checkbox"/> Blood infection (sepsis) <input type="checkbox"/> Urinary tract infection <input type="checkbox"/> Other.....	Antibiotics through the vein <input type="checkbox"/> Oral antibiotics <input type="checkbox"/> Nebuliser <input type="checkbox"/> Fluids through the vein <input type="checkbox"/> Other.....
2 nd Admission Name of hospital Date of Admission:		Meningitis <input type="checkbox"/> Pneumonia <input type="checkbox"/> Bronchiolitis <input type="checkbox"/> Wheeze <input type="checkbox"/> Blood infection (sepsis) <input type="checkbox"/> Urinary tract infection <input type="checkbox"/> Other.....	Antibiotics through the vein <input type="checkbox"/> Oral antibiotics <input type="checkbox"/> Nebuliser <input type="checkbox"/> Fluids through the vein <input type="checkbox"/> Other.....
3 rd Admission Name of hospital Date of Admission:		Meningitis <input type="checkbox"/> Pneumonia <input type="checkbox"/> Bronchiolitis <input type="checkbox"/> Wheeze <input type="checkbox"/> Blood infection (sepsis) <input type="checkbox"/> Urinary tract infection <input type="checkbox"/> Other.....	Antibiotics through the vein <input type="checkbox"/> Oral antibiotics <input type="checkbox"/> Nebuliser <input type="checkbox"/> Fluids through the vein <input type="checkbox"/> Other.....

(Please find the attached tables if needed for more admission)

GP Visit

1. Has your child ever visited the GP when unwell?

Yes/No

- If YES, How many times

(Please fill this table for further information about your child's GP visit)

Date/age of visit	Symptoms	Diagnosis	Treatment
	Cough <input type="checkbox"/> Runny/blocked nose <input type="checkbox"/> Rash <input type="checkbox"/> Temperature <input type="checkbox"/> Other.....	Viral cold <input type="checkbox"/> Chest infection <input type="checkbox"/> Bronchiolitis <input type="checkbox"/> Wheeze <input type="checkbox"/> Feeding problems <input type="checkbox"/> Urinary tract infection <input type="checkbox"/> Ear infection <input type="checkbox"/> Other.....	Advice only <input type="checkbox"/> Oral antibiotics <input type="checkbox"/> Inhalers <input type="checkbox"/> Paracetamol/Calpol <input type="checkbox"/> Ibuprofen/Nurofen <input type="checkbox"/> Other.....
	Cough <input type="checkbox"/> Runny/blocked nose <input type="checkbox"/> Rash <input type="checkbox"/> Temperature <input type="checkbox"/> Other.....	Viral cold <input type="checkbox"/> Chest infection <input type="checkbox"/> Bronchiolitis <input type="checkbox"/> Wheeze <input type="checkbox"/> Feeding problems <input type="checkbox"/> Urinary tract infection <input type="checkbox"/> Ear infection <input type="checkbox"/> Other.....	Advice only <input type="checkbox"/> Oral antibiotics <input type="checkbox"/> Inhalers <input type="checkbox"/> Paracetamol/Calpol <input type="checkbox"/> Ibuprofen/Nurofen <input type="checkbox"/> Other.....
	Cough <input type="checkbox"/> Runny/blocked nose <input type="checkbox"/> Rash <input type="checkbox"/> Temperature <input type="checkbox"/> Other.....	Viral cold <input type="checkbox"/> Chest infection <input type="checkbox"/> Bronchiolitis <input type="checkbox"/> Wheeze <input type="checkbox"/> Feeding problems <input type="checkbox"/> Urinary tract infection <input type="checkbox"/> Ear infection <input type="checkbox"/> Other.....	Advice only <input type="checkbox"/> Oral antibiotics <input type="checkbox"/> Inhalers <input type="checkbox"/> Paracetamol/Calpol <input type="checkbox"/> Ibuprofen/Nurofen <input type="checkbox"/> Other.....
	Cough <input type="checkbox"/> Runny/blocked nose <input type="checkbox"/> Rash <input type="checkbox"/> Temperature <input type="checkbox"/> Other.....	Viral cold <input type="checkbox"/> Chest infection <input type="checkbox"/> Bronchiolitis <input type="checkbox"/> Wheeze <input type="checkbox"/> Feeding problems <input type="checkbox"/> Urinary tract infection <input type="checkbox"/> Ear infection <input type="checkbox"/> Other.....	Advice only <input type="checkbox"/> Oral antibiotics <input type="checkbox"/> Inhalers <input type="checkbox"/> Paracetamol/Calpol <input type="checkbox"/> Ibuprofen/Nurofen <input type="checkbox"/> Other.....

(Please find the attached tables if needed for more GP visit)

Child's Health at Home

2. Are your child's vaccinations up to date?

☐ Yes

☐ No

☐ Partly

- Has your child been unwell at home but not needed to go to the GP's? Yes/ No

If Yes, Please tell us more about it.

Date or age of illness	Symptoms	Treatment
	Cough <input type="checkbox"/> Runny/blocked nose <input type="checkbox"/> Rash <input type="checkbox"/> Temperature <input type="checkbox"/> Diarrhoea/vomiting <input type="checkbox"/> Other.....	Observe only <input type="checkbox"/> Inhalers <input type="checkbox"/> Paracetamol (Calpol) <input type="checkbox"/> And/or Ibuprofen (Nurofen) <input type="checkbox"/> Other.....
	Cough <input type="checkbox"/> Runny/blocked nose <input type="checkbox"/> Rash <input type="checkbox"/> Temperature <input type="checkbox"/> Diarrhoea/vomiting <input type="checkbox"/> Other.....	Observe only <input type="checkbox"/> Inhalers <input type="checkbox"/> Paracetamol (Calpol) <input type="checkbox"/> And/or Ibuprofen (Nurofen) <input type="checkbox"/> Other.....
	Cough <input type="checkbox"/> Runny/blocked nose <input type="checkbox"/> Rash <input type="checkbox"/> Temperature <input type="checkbox"/> Diarrhoea/vomiting <input type="checkbox"/> Other.....	Observe only <input type="checkbox"/> Inhalers <input type="checkbox"/> Paracetamol (Calpol) <input type="checkbox"/> And/or Ibuprofen (Nurofen) <input type="checkbox"/> Other.....
	Cough <input type="checkbox"/> Runny/blocked nose <input type="checkbox"/> Rash <input type="checkbox"/> Temperature <input type="checkbox"/> Diarrhoea/vomiting <input type="checkbox"/> Other.....	Observe only <input type="checkbox"/> Inhalers <input type="checkbox"/> Paracetamol (Calpol) <input type="checkbox"/> And/or Ibuprofen (Nurofen) <input type="checkbox"/> Other.....

(Please find the attached tables if needed for more Child's Health at Home)

Did you have problems understanding this questionnaire?

Yes ☐ no ☐

Please write any comments you have about your child's health or about the questionnaire in the space below:

.....
.....
.....

Thank you for completing the questionnaire. It will cost you nothing to return it if you use the pre-paid envelope provided (FREEPOST). No stamp required.

For any queries please do not hesitate to contact us:

Dr Kaninika Basu

01273 696955, ext 2404

Questions about Infection

Hospital Admissions

Was your baby admitted to the Special Care Baby Unit? Yes/No

- If YES why?

☐ Premature

☐ Breathing problems

☐ Suspected infection

☐ Confirmed infection

- If infection what kind? ☐ Chest ☐ Meningitis ☐ Blood ☐ Other?

.....

- Has your child had any/other admissions to hospital? Yes/No

If YES, How many times?

Please fill this table for further information about your child hospital admission

Name of hospital and Date of admission	Age	Diagnosis	Treatment
1 st Admission Name of hospital Date of Admission:		Meningitis <input type="checkbox"/> Pneumonia <input type="checkbox"/> Bronchiolitis <input type="checkbox"/> Wheeze <input type="checkbox"/> Blood infection (sepsis) <input type="checkbox"/> Urinary tract infection <input type="checkbox"/> Other.....	Antibiotics through the vein <input type="checkbox"/> Oral antibiotics <input type="checkbox"/> Nebuliser <input type="checkbox"/> Fluids through the vein <input type="checkbox"/> Other.....
2 nd Admission Name of hospital Date of Admission:		Meningitis <input type="checkbox"/> Pneumonia <input type="checkbox"/> Bronchiolitis <input type="checkbox"/> Wheeze <input type="checkbox"/> Blood infection (sepsis) <input type="checkbox"/> Urinary tract infection <input type="checkbox"/> Other.....	Antibiotics through the vein <input type="checkbox"/> Oral antibiotics <input type="checkbox"/> Nebuliser <input type="checkbox"/> Fluids through the vein <input type="checkbox"/> Other.....
3 rd Admission Name of hospital Date of Admission:		Meningitis <input type="checkbox"/> Pneumonia <input type="checkbox"/> Bronchiolitis <input type="checkbox"/> Wheeze <input type="checkbox"/> Blood infection (sepsis) <input type="checkbox"/> Urinary tract infection <input type="checkbox"/> Other.....	Antibiotics through the vein <input type="checkbox"/> Oral antibiotics <input type="checkbox"/> Nebuliser <input type="checkbox"/> Fluids through the vein <input type="checkbox"/> Other.....

(Please find the attached tables if needed for more admission)

GP Visit

3. Has your child ever visited the GP when unwell?

Yes/No

- If YES, How many times

(Please fill this table for further information about your child's GP visit)

Date/age of visit	Symptoms	Diagnosis	Treatment
	Cough <input type="checkbox"/> Runny/blocked nose <input type="checkbox"/> Rash <input type="checkbox"/> Temperature <input type="checkbox"/> Other.....	Viral cold <input type="checkbox"/> Chest infection <input type="checkbox"/> Bronchiolitis <input type="checkbox"/> Wheeze <input type="checkbox"/> Feeding problems <input type="checkbox"/> Urinary tract infection <input type="checkbox"/> Ear infection <input type="checkbox"/> Other.....	Advice only <input type="checkbox"/> Oral antibiotics <input type="checkbox"/> Inhalers <input type="checkbox"/> Paracetamol/Calpol <input type="checkbox"/> Ibuprofen/Nurofen <input type="checkbox"/> Other.....
	Cough <input type="checkbox"/> Runny/blocked nose <input type="checkbox"/> Rash <input type="checkbox"/> Temperature <input type="checkbox"/> Other.....	Viral cold <input type="checkbox"/> Chest infection <input type="checkbox"/> Bronchiolitis <input type="checkbox"/> Wheeze <input type="checkbox"/> Feeding problems <input type="checkbox"/> Urinary tract infection <input type="checkbox"/> Ear infection <input type="checkbox"/> Other.....	Advice only <input type="checkbox"/> Oral antibiotics <input type="checkbox"/> Inhalers <input type="checkbox"/> Paracetamol/Calpol <input type="checkbox"/> Ibuprofen/Nurofen <input type="checkbox"/> Other.....
	Cough <input type="checkbox"/> Runny/blocked nose <input type="checkbox"/> Rash <input type="checkbox"/> Temperature <input type="checkbox"/> Other.....	Viral cold <input type="checkbox"/> Chest infection <input type="checkbox"/> Bronchiolitis <input type="checkbox"/> Wheeze <input type="checkbox"/> Feeding problems <input type="checkbox"/> Urinary tract infection <input type="checkbox"/> Ear infection <input type="checkbox"/> Other.....	Advice only <input type="checkbox"/> Oral antibiotics <input type="checkbox"/> Inhalers <input type="checkbox"/> Paracetamol/Calpol <input type="checkbox"/> Ibuprofen/Nurofen <input type="checkbox"/> Other.....
	Cough <input type="checkbox"/> Runny/blocked nose <input type="checkbox"/> Rash <input type="checkbox"/> Temperature <input type="checkbox"/> Other.....	Viral cold <input type="checkbox"/> Chest infection <input type="checkbox"/> Bronchiolitis <input type="checkbox"/> Wheeze <input type="checkbox"/> Feeding problems <input type="checkbox"/> Urinary tract infection <input type="checkbox"/> Ear infection <input type="checkbox"/> Other.....	Advice only <input type="checkbox"/> Oral antibiotics <input type="checkbox"/> Inhalers <input type="checkbox"/> Paracetamol/Calpol <input type="checkbox"/> Ibuprofen/Nurofen <input type="checkbox"/> Other.....

(Please find the attached tables if needed for more GP visit)

Child's Health at Home

4. Are your child's vaccinations up to date?

☐ Yes

☐ No

☐ Partly

• Has your child been unwell at home but not needed to go to the GP's? Yes/ No

If Yes, Please tell us more about it.

Date or age of illness	Symptoms	Treatment
	Cough <input type="checkbox"/> Runny/blocked nose <input type="checkbox"/> Rash <input type="checkbox"/> Temperature <input type="checkbox"/> Diarrhoea/vomiting <input type="checkbox"/> Other.....	Observe only <input type="checkbox"/> Inhalers <input type="checkbox"/> Paracetamol (Calpol) <input type="checkbox"/> And/or Ibuprofen (Nurofen) <input type="checkbox"/> Other.....
	Cough <input type="checkbox"/> Runny/blocked nose <input type="checkbox"/> Rash <input type="checkbox"/> Temperature <input type="checkbox"/> Diarrhoea/vomiting <input type="checkbox"/> Other.....	Observe only <input type="checkbox"/> Inhalers <input type="checkbox"/> Paracetamol (Calpol) <input type="checkbox"/> And/or Ibuprofen (Nurofen) <input type="checkbox"/> Other.....
	Cough <input type="checkbox"/> Runny/blocked nose <input type="checkbox"/> Rash <input type="checkbox"/> Temperature <input type="checkbox"/> Diarrhoea/vomiting <input type="checkbox"/> Other.....	Observe only <input type="checkbox"/> Inhalers <input type="checkbox"/> Paracetamol (Calpol) <input type="checkbox"/> And/or Ibuprofen (Nurofen) <input type="checkbox"/> Other.....
	Cough <input type="checkbox"/> Runny/blocked nose <input type="checkbox"/> Rash <input type="checkbox"/> Temperature <input type="checkbox"/> Diarrhoea/vomiting <input type="checkbox"/> Other.....	Observe only <input type="checkbox"/> Inhalers <input type="checkbox"/> Paracetamol (Calpol) <input type="checkbox"/> And/or Ibuprofen (Nurofen) <input type="checkbox"/> Other.....

(Please find the attached tables if needed for more Child's Health at Home)

The Influence of genetic and environmental factors on childhood diseases

Postal questionnaire to assess asthma/allergy: Year 1

Thank you for agreeing to participate in our study. Please fill out this questionnaire with the information about the first 1 year of your child's life and return it in the pre-paid envelope that has been supplied.

How to complete the questionnaire: Please tick the appropriate box

Example: Person completing questionnaire (tick box please):

Mother ☒ Father ☐ Other ☐

Name of Child:

Bar Code Sticker

Date of Birth:

- Person completing questionnaire (tick box please):

Mother ☐ Father ☐ Other ☐

- Date questionnaire completed: day ____ month ____ year ____
(please fill in today's date)

1. In the last year, has your child had an **ITCHY** skin condition - by *itchy* we mean scratching or rubbing the skin)?

Yes	<input type="checkbox"/>
No	<input type="checkbox"/>

IF YOU HAVE ANSWERED 'NO' PLEASE SKIP TO QUESTION 2

IF YOU HAVE ANSWERED 'YES' PLEASE ANSWER THE QUESTIONS IN THE SHADED BOX BELOW:

1b. Was this ITCHY skin condition coming and going for at least six months?

Yes	<input type="checkbox"/>
No	<input type="checkbox"/>

1c. Has your child had this ITCHY skin condition in the last week?

Yes	<input type="checkbox"/>
No	<input type="checkbox"/>

1d. How old was your child when this skin condition began? months old

1e. Has this skin condition ever affected the skin creases in the past – by skin creases we mean fronts of elbows, behind the knees, front of ankles, around the neck or around the eyes?

Yes	<input type="checkbox"/>
No	<input type="checkbox"/>

2. In the first year, has your child suffered from a dry skin in general?

Yes	<input type="checkbox"/>
No	<input type="checkbox"/>

3. In the first year, has your child suffered from any of the following skin complaints: (PLEASE TICK ALL THAT APPLY).

Eczema	<input type="checkbox"/>
Facial spots	<input type="checkbox"/>
Nappy rash	<input type="checkbox"/>

4. In the first year, has your child ever had wheezing or whistling in the chest? By “wheezing” we mean breathing that makes a high-pitched whistling or squeaking sound from the chest, not the throat

Yes	<input type="checkbox"/>
No	<input type="checkbox"/>

IF YOU HAVE ANSWERED 'NO' PLEASE SKIP TO QUESTION 12

IF YOU HAVE ANSWERED 'YES' PLEASE ANSWER THE QUESTIONS IN THE SHADED BOX BELOW:

4a. How old was your child when he/she first began to wheeze? months

5. In the first year, has your child had wheezing or whistling in the chest during or soon after a cold or flu? Yes ☐
No ☐

6. How many attacks of wheezing has your child had in the first year? None ☐
1 to 3 ☐
4 to 12 ☐
More than 12 ☐

7. Do these attacks cause him/her to be short of breath? Yes, always ☐
Most of the time ☐
Occasionally ☐
No, never ☐

8. Which of these two descriptions fits best your child's wheeze? (TICK ONE ONLY)

My child has only short attacks of wheeze, for example with colds. In between these attacks, he/she does not normally wheeze ☐
My child wheezes always or a lot of the time. With colds he/she has attacks with more severe wheeze ☐

9. In the first year, how often, on average, has your child's sleep been disturbed due to wheezing? never woken with wheezing ☐
less than one night per week ☐
one or more nights per week ☐

10. In the first year, did any of the following things cause wheezing in your child?

Feeding; playing; exercise?

Yes ☐

No ☐

Don't know ☐

laughing, crying or excitement?

Yes ☐

No ☐

Don't know ☐

Contact with pets or other animals?

Yes ☐

No ☐

Don't know ☐

Food or drinks?

Yes ☐

No ☐

Don't know ☐

11. Looking back on the first year, do you think that your child had asthma?

Yes ☐

No ☐

12. Does your child usually have a cough with colds?

Yes ☐

No ☐

13. Does your child have a cough even without having a cold?

Yes, always ☐

Yes, sometimes ☐

No, never ☐

14. Do you think that your child coughs more than other children?

Yes ☐

No ☐

15. In the first year, has your child had a dry cough at night, apart from a cough associated with a cold or a chest infection?

Yes ☐

No ☐

16. In the first year, did the following things cause coughing in your child?

Feeding, playing or exercise?

Yes	<input type="checkbox"/>
No	<input type="checkbox"/>
Don't know	<input type="checkbox"/>

laughing, crying or excitement?

Yes	<input type="checkbox"/>
No	<input type="checkbox"/>
Don't know	<input type="checkbox"/>

contact with pets or other animals?

Yes	<input type="checkbox"/>
No	<input type="checkbox"/>
Don't know	<input type="checkbox"/>

food or drinks?

Yes	<input type="checkbox"/>
No	<input type="checkbox"/>
Don't know	<input type="checkbox"/>

17. How often did your child see the GP for coughing or wheezing during the first 12months?

Never	<input type="checkbox"/>
Once	<input type="checkbox"/>
2-3 times	<input type="checkbox"/>
4-6 times	<input type="checkbox"/>
7 or more times	<input type="checkbox"/>

18. In the first 12 months, has wheezing or asthma resulted in your child:

being referred to a consultant in hospital

Yes	<input type="checkbox"/>
No	<input type="checkbox"/>

being admitted to hospital

Yes	<input type="checkbox"/>
No	<input type="checkbox"/>

attending the casualty (A and E) department

Yes	<input type="checkbox"/>
No	<input type="checkbox"/>

attending (or calling) the GP in an emergency

Yes	<input type="checkbox"/>
No	<input type="checkbox"/>

Yes	<input type="checkbox"/>
-----	--------------------------

19. Did your child take any of the following drugs in the first 12 months?
 Salbutamol, Ventolin, Bricanyl or other blue inhaler

No ☐
 Don't know ☐

Pulmicort, Flixotide, Becotide or other brown inhaler

Yes ☐
 No ☐
 Don't know ☐

Steroid tablets (prednisolone) for asthma attacks

Yes ☐
 No ☐
 Don't know ☐

20. In the first year, did your child suffer from rattly breathing (rattles)?

Never ☐
 Only with a cold ☐
 Sometimes even without a cold ☐
 Almost always ☐

21. In the first year, how many times has your child had a cold or flu?

Never ☐
 1-3 times ☐
 4-6 times ☐
 7-10 times ☐
 More than 10 times ☐

22. How long does a cold usually last in your child?

Less than 1 week ☐
 1 to 2 weeks ☐
 2 to 4 weeks ☐
 More than 4 weeks ☐

23. In the first year, has your child had a problem with sneezing, or a runny, or blocked nose when he/she did NOT have a cold or the flu?

Yes ☐
 No ☐

24. In the first year, how much did this nose problem interfere with your child's feeding, playing or other activities?

Not at all ☐
 A little ☐
 A moderate amount ☐
 A lot ☐

25. Over the first 12months, has your child snored at night?

Yes	<input type="checkbox"/>
No	<input type="checkbox"/>

IF YOU HAVE ANSWERED 'NO' PLEASE SKIP TO QUESTION 26

IF YOU HAVE ANSWERED 'YES' PLEASE ANSWER THE QUESTIONS IN THE SHADED BOX BELOW:

25.a. If yes, has he/she snored:	Only with a cold	<input type="checkbox"/>
	Sometimes even without a cold	<input type="checkbox"/>
	Almost always	<input type="checkbox"/>
25.b Did the snoring disturb your child's sleep?	Not at all	<input type="checkbox"/>
	A little	<input type="checkbox"/>
	A moderate amount	<input type="checkbox"/>
	A lot	<input type="checkbox"/>

26. In the first 12months, has your child had any ear infections?

No, never	<input type="checkbox"/>
Yes, once	<input type="checkbox"/>
Yes, more than once	<input type="checkbox"/>

27. Has your child ever suffered from any of the following conditions?

pneumonia?

No, never	<input type="checkbox"/>
Yes, once	<input type="checkbox"/>
Yes, more than once	<input type="checkbox"/>

whooping cough?

No, never	<input type="checkbox"/>
Yes, once	<input type="checkbox"/>
Yes, more than once	<input type="checkbox"/>

bronchiolitis?

No, never	<input type="checkbox"/>
Yes, once	<input type="checkbox"/>
Yes, more than once	<input type="checkbox"/>

croup?

No, never	<input type="checkbox"/>
Yes, once	<input type="checkbox"/>
Yes, more than once	<input type="checkbox"/>

28. Does your child attend day care, childminder, nursery school or play school?

Yes	<input type="checkbox"/>
No	<input type="checkbox"/>

29. Was your child breastfed?

Yes	<input type="checkbox"/>
No	<input type="checkbox"/>

If yes, how long:

less than a month	<input type="checkbox"/>
1-3 months	<input type="checkbox"/>
4-6 months	<input type="checkbox"/>
more than 6 months	<input type="checkbox"/>

30. During the first year of life, did your child posit or vomit?

Not at all	<input type="checkbox"/>
A little	<input type="checkbox"/>
A lot	<input type="checkbox"/>

31. Do you think your child has a reaction to any food items?

Yes	<input type="checkbox"/>
No	<input type="checkbox"/>

IF YOU HAVE ANSWERED 'NO' PLEASE SKIP TO QUESTION 32

IF YOU HAVE ANSWERED 'YES' PLEASE ANSWER THE QUESTIONS IN THE SHADED BOX BELOW:

31a. Does your child have a reaction to any of these foods? (PLEASE TICK ALL THAT APPLY)	Peanuts	<input type="checkbox"/>
	Cows milk	<input type="checkbox"/>
	Egg	<input type="checkbox"/>
If you have ticked 'other', please describe the type of food that causes the reaction:.....	Gluten (eg wheat, oats)	<input type="checkbox"/>
	Fruit	<input type="checkbox"/>
	Other (please describe)	<input type="checkbox"/>
31b. What type of reaction does the food cause? (PLEASE TICK ALL THAT APPLY)	Breathing problems	<input type="checkbox"/>
	Vomit	<input type="checkbox"/>
	Diarrhea	<input type="checkbox"/>
If you have ticked 'other', please describe the type of reaction:.....	Stomach pain	<input type="checkbox"/>
	Rashes	<input type="checkbox"/>
	Irritability	<input type="checkbox"/>
	Other (please describe)	<input type="checkbox"/>

31c. Has your child been treated by a doctor for allergies to any of these foods? (PLEASE TICK ALL THAT APPLY)

If you have ticked 'other', please describe the type of food allergy that has been treated:.....
.....

Peanuts	<input type="checkbox"/>
Cows milk	<input type="checkbox"/>
Egg	<input type="checkbox"/>
Gluten (eg wheat, oats)	<input type="checkbox"/>
Fruit	<input type="checkbox"/>
Other (please describe)	<input type="checkbox"/>

32. Does your child have brothers and sisters who have the same mother and father as him/her?

Yes	<input type="checkbox"/>
No	<input type="checkbox"/>

If yes, how many? (please fill in number)

If yes, how many have: -

Asthma or wheezing? (please fill in number)	<input type="text"/>
Hay fever? (please fill in number)	<input type="text"/>
Eczema? (please fill in number)	<input type="text"/>

33. How many children under 16 live in your household?

(PLEASE FILL IN NUMBER)

34. How many adults over 16 usually live in your household?

(PLEASE FILL IN NUMBER)

35. How many rooms are there in your house, not counting kitchens, bathrooms and toilets? (PLEASE FILL IN NUMBER)

36. At what age did the child's mother finish full-time education?
(PLEASE FILL IN AGE)

37. Which fuel is mainly used for cooking in your home?

Electricity	<input type="checkbox"/>
Gas	<input type="checkbox"/>
Other fuel	<input type="checkbox"/>

38. How do you heat your home? (PLEASE
TICK AS MANY AS APPLY)

Electric central heating	<input type="checkbox"/>
Gas central heating	<input type="checkbox"/>
Central heating with other fuel, e.g. oil	<input type="checkbox"/>
Heaters in rooms	<input type="checkbox"/>
Coal or wood fire	<input type="checkbox"/>

39. Is there visible damp within the house?

Yes	<input type="checkbox"/>
No	<input type="checkbox"/>

If there is visible damp, which rooms is it in?
(PLEASE TICK ALL THAT APPLY)

Kitchen	<input type="checkbox"/>
Bathroom	<input type="checkbox"/>
Child's bedroom	<input type="checkbox"/>
Other living areas	<input type="checkbox"/>

40. What type of flooring does your child have in his/her bedroom?

Carpet	<input type="checkbox"/>
Laminate	<input type="checkbox"/>
Laminate with rug	<input type="checkbox"/>
Other hard flooring	<input type="checkbox"/>
Other	<input type="checkbox"/>

41. Is your child exposed to animals?

(By exposed we mean do they come into close
contact with any animals on a regular basis)

Yes	<input type="checkbox"/>
No	<input type="checkbox"/>

If yes, which of the following animals?

(PLEASE TICK ALL THAT APPLY)

If your child is exposed to other animals that are not on the list,
please write which kind of animals in the space below:

.....

Cat	<input type="checkbox"/>
Dog	<input type="checkbox"/>
Bird	<input type="checkbox"/>
Fish	<input type="checkbox"/>
Rat	<input type="checkbox"/>
Gerbil	<input type="checkbox"/>
Rabbit	<input type="checkbox"/>
Hamster	<input type="checkbox"/>
Guinea pig	<input type="checkbox"/>
Sheep	<input type="checkbox"/>
Pigs	<input type="checkbox"/>
Cows	<input type="checkbox"/>
Horses	<input type="checkbox"/>

42. Does the child's mother smoke cigarettes?

Yes	<input type="checkbox"/>
No	<input type="checkbox"/>

If yes, how many per day?

1 to 10	<input type="checkbox"/>
11 to 20	<input type="checkbox"/>
More than 20	<input type="checkbox"/>

43. Do any other household members smoke cigarettes?

Yes	<input type="checkbox"/>
No	<input type="checkbox"/>

If yes, how many per day (total cigarettes smoked by
household members other than mother)?

1 to 10	<input type="checkbox"/>
11 to 20	<input type="checkbox"/>
More than 20	<input type="checkbox"/>

44. How would you best describe
the location of your house?

(PLEASE TICK THE ONE THAT
BEST APPLIES)

In a street with very dense traffic (main road)	<input type="checkbox"/>
In a street with moderate traffic (residential road)	<input type="checkbox"/>
In a quiet street with little or no traffic	<input type="checkbox"/>

45. Did you have any problems understanding this questionnaire?

Yes	<input type="checkbox"/>
No	<input type="checkbox"/>

Please write any comments you have about your child's health or about the questionnaire in the
space below:

.....
.....
.....
Thank you for completing the questionnaire. It will cost you nothing to return it if you use the pre-paid envelope provided.

For any queries please do not hesitate to contact us:

Ms Liz Lance, Dr Kaninika Basu; Contact: 01273 696955, ext 2404, 2353

Liz.Lance@bsuh.nhs.uk, k.basu@bsms.ac.uk

The Influence of genetic and environmental factors on childhood diseases

Postal questionnaire to assess asthma/allergy: Year 2

Thank you for agreeing to participate in our study. Please fill out this questionnaire with the information about the first 2 years of your child's life and return it in the pre-paid envelope that has been supplied.

How to complete the questionnaire: Please tick the appropriate box

Example: Person completing questionnaire (tick box please):

Mother ☒ Father ☐ Other ☐

Name of Child:

Bar Code Sticker

Date of Birth:

- Person completing questionnaire (tick box please):

Mother ☐ Father ☐ Other ☐

- Date questionnaire completed: day ____ month ____ year ____
(please fill in today's date)

1. In the last year, has your child had an **ITCHY** skin condition - by *itchy* we mean scratching or rubbing the skin)?

Yes	<input type="checkbox"/>
No	<input type="checkbox"/>

IF YOU HAVE ANSWERED 'NO' PLEASE SKIP TO QUESTION 2

IF YOU HAVE ANSWERED 'YES' PLEASE ANSWER THE QUESTIONS IN THE SHADED BOX BELOW:

1b. Was this ITCHY skin condition coming and going for at least six months?

Yes	<input type="checkbox"/>
No	<input type="checkbox"/>

1c. Has your child had this ITCHY skin condition in the last week?

Yes	<input type="checkbox"/>
No	<input type="checkbox"/>

1d. How old was your child when this skin condition began?

<input type="text"/>	months old
----------------------	------------

1e. Has this skin condition ever affected the skin creases in the past – by skin creases we mean fronts of elbows, behind the knees, front of ankles, around the neck or around the eyes?

Yes	<input type="checkbox"/>
No	<input type="checkbox"/>

2. Between 12 and 24months, has your child suffered from a dry skin in general?

Yes	<input type="checkbox"/>
No	<input type="checkbox"/>

3. Between 12 and 24months, has your child suffered from any of the following skin complaints: (PLEASE TICK ALL THAT APPLY).

Eczema	<input type="checkbox"/>
Facial spots	<input type="checkbox"/>
Nappy rash	<input type="checkbox"/>

4. Between 12 and 24months, has your child ever had wheezing or whistling in the chest? By “wheezing” we mean breathing that makes a high-pitched whistling or squeaking sound from the chest, not the throat

Yes	<input type="checkbox"/>
No	<input type="checkbox"/>

IF YOU HAVE ANSWERED 'NO' PLEASE SKIP TO QUESTION 12

IF YOU HAVE ANSWERED 'YES' PLEASE ANSWER THE QUESTIONS IN THE SHADED BOX BELOW:

4a. How old was your child when he/she first began to wheeze? months

5. In the second year, has your child had wheezing or whistling in the chest during or soon after a cold or flu? Yes ☐
No ☐

6. How many attacks of wheezing has your child had between 12 and 24months? None ☐
1 to 3 ☐
4 to 12 ☐
More than 12 ☐

7. Do these attacks cause him/her to be short of breath? Yes, always ☐
Most of the time ☐
Occasionally ☐
No, never ☐

8. Which of these two descriptions fits best your child's wheeze? (TICK ONE ONLY)

My child has only short attacks of wheeze, for example with colds. In between these attacks, he/she does not normally wheeze ☐
My child wheezes always or a lot of the time. With colds he/she has attacks with more severe wheeze ☐

9. Between 12 and 24months, how often, on average, has your child's sleep been disturbed due to wheezing? never woken with wheezing ☐
less than one night per week ☐
one or more nights per week ☐

10. Between 12 and 24months, did any of the following things cause wheezing in your child?

Feeding; playing; exercise?

Yes

☐

No

☐

Don't know

☐

laughing, crying or excitement?

Yes

☐

No

☐

Don't know

☐

Contact with pets or other animals?

Yes

☐

No

☐

Don't know

☐

Food or drinks?

Yes

☐

No

☐

Don't know

☐

11. Looking back between 12 and 24months, do you think that your child had asthma?

Yes

☐

No

☐

12. Does your child usually have a cough with colds?

Yes

☐

No

☐

13. Does your child have a cough even without having a cold?

Yes, always

☐

Yes, sometimes

☐

No, never

☐

14. Do you think that your child coughs more than other children?

Yes

☐

No

☐

15. Between 12 and 24months, has your child had a dry cough at night, apart from a cough associated with a cold or a chest infection?

Yes

☐

No

☐

16. Between 12 and 24months, did the following things cause coughing in your child?

Feeding, playing or exercise?

Yes
No
Don't know

laughing, crying or excitement?

Yes
No
Don't know

contact with pets or other animals?

Yes
No
Don't know

food or drinks?

Yes
No
Don't know

17. How often did your child see the GP for coughing or wheezing between 12 and 24months?

Never
Once
2-3 times
4-6 times
7 or more times

18. Between 12 and 24months, has wheezing or asthma resulted in your child:

being referred to a consultant in hospital

Yes
No

being admitted to hospital

Yes
No

attending the casualty (A and E) department

Yes
No

attending (or calling) the GP in an emergency

Yes
No

Yes

19. Did your child take any of the following drugs between 12 and 24months? No ☐
Salbutamol, Ventolin, Bricanyl or other blue inhaler Don't know ☐

Pulmicort, Flixotide, Becotide or other brown inhaler

Yes ☐
 No ☐
 Don't know ☐

Steroid tablets (prednisolone) for asthma attacks

Yes ☐
 No ☐
 Don't know ☐

20. Between 12 and 24months, did your child suffer from rattly breathing (rattles)?

Never ☐
 Only with a cold ☐
 Sometimes even without a cold ☐
 Almost always ☐

21. In the second year, how many times has your child had a cold or flu?

Never ☐
 1-3 times ☐
 4-6 times ☐
 7-10 times ☐
 More than 10 times ☐

22. How long does a cold usually last in your child?

Less than 1 week ☐
 1 to 2 weeks ☐
 2 to 4 weeks ☐
 More than 4 weeks ☐

23. Between 12 and 24months, has your child had a problem with sneezing, or a runny, or blocked nose when he/she did NOT have a cold or the flu?

Yes ☐
 No ☐

24. Between 12 and 24months, how much did this nose problem interfere with your child's feeding, playing or other activities?

Not at all ☐
 A little ☐
 A moderate amount ☐
 A lot ☐

25. Between 12 and 24months, has your child snored at night?

Yes	<input type="checkbox"/>
No	<input type="checkbox"/>

IF YOU HAVE ANSWERED 'NO' PLEASE SKIP TO QUESTION 26

IF YOU HAVE ANSWERED 'YES' PLEASE ANSWER THE QUESTIONS IN THE SHADED BOX BELOW:

25.a. If yes, has he/she snored:	Only with a cold	<input type="checkbox"/>
	Sometimes even without a cold	<input type="checkbox"/>
	Almost always	<input type="checkbox"/>
25.b Did the snoring disturb your child's sleep?	Not at all	<input type="checkbox"/>
	A little	<input type="checkbox"/>
	A moderate amount	<input type="checkbox"/>
	A lot	<input type="checkbox"/>

26. Between 12 and 24months, has your child had any ear infections?

No, never	<input type="checkbox"/>
Yes, once	<input type="checkbox"/>
Yes, more than once	<input type="checkbox"/>

27. Has your child ever suffered from any of the following conditions?

pneumonia?

No, never	<input type="checkbox"/>
Yes, once	<input type="checkbox"/>
Yes, more than once	<input type="checkbox"/>

whooping cough?

No, never	<input type="checkbox"/>
Yes, once	<input type="checkbox"/>
Yes, more than once	<input type="checkbox"/>

bronchiolitis?

No, never	<input type="checkbox"/>
Yes, once	<input type="checkbox"/>
Yes, more than once	<input type="checkbox"/>

croup?

No, never	<input type="checkbox"/>
Yes, once	<input type="checkbox"/>
Yes, more than once	<input type="checkbox"/>

28. Does your child attend day care, childminder, nursery school or play school?

Yes	<input type="checkbox"/>
No	<input type="checkbox"/>

29. Was your child breastfed?

Yes	<input type="checkbox"/>
No	<input type="checkbox"/>

If yes, how long:

less than a month	<input type="checkbox"/>
1-3 months	<input type="checkbox"/>
4-6 months	<input type="checkbox"/>
more than 6 months	<input type="checkbox"/>

30. Between 12 and 24 months, did your child posit or vomit?

Not at all	<input type="checkbox"/>
A little	<input type="checkbox"/>
A lot	<input type="checkbox"/>

31. Do you think your child has a reaction to any food items?

Yes	<input type="checkbox"/>
No	<input type="checkbox"/>

IF YOU HAVE ANSWERED 'NO' PLEASE SKIP TO QUESTION 32

IF YOU HAVE ANSWERED 'YES' PLEASE ANSWER THE QUESTIONS IN THE SHADED BOX BELOW:

31a. Does your child have a reaction to any of these foods? (PLEASE TICK ALL THAT APPLY)	Peanuts	<input type="checkbox"/>
	Cows milk	<input type="checkbox"/>
	Egg	<input type="checkbox"/>
If you have ticked 'other', please describe the type of food that causes the reaction:.....	Gluten (eg wheat, oats)	<input type="checkbox"/>
	Fruit	<input type="checkbox"/>
	Other (please describe)	<input type="checkbox"/>
31b. What type of reaction does the food cause? (PLEASE TICK ALL THAT APPLY)	Breathing problems	<input type="checkbox"/>
	Vomit	<input type="checkbox"/>
	Diarrhea	<input type="checkbox"/>
If you have ticked 'other', please describe the type of reaction:.....	Stomach pain	<input type="checkbox"/>
	Rashes	<input type="checkbox"/>
	Irritability	<input type="checkbox"/>
	Other (please describe)	<input type="checkbox"/>

31c. Has your child been treated by a doctor for allergies to any of these foods? (PLEASE TICK ALL THAT APPLY)

If you have ticked 'other', please describe the type of food allergy that has been treated:.....
.....

Peanuts	<input type="checkbox"/>
Cows milk	<input type="checkbox"/>
Egg	<input type="checkbox"/>
Gluten (eg wheat, oats)	<input type="checkbox"/>
Fruit	<input type="checkbox"/>
Other (please describe)	<input type="checkbox"/>

32. Does your child have brothers and sisters who have the same mother and father as him/her?

Yes	<input type="checkbox"/>
No	<input type="checkbox"/>

If yes, how many? (please fill in number)

If yes, how many have: -

Asthma or wheezing? (please fill in number)	<input type="text"/>
Hay fever? (please fill in number)	<input type="text"/>
Eczema? (please fill in number)	<input type="text"/>

33. How many children under 16 live in your household?

(PLEASE FILL IN NUMBER)

34. How many adults over 16 usually live in your household?

(PLEASE FILL IN NUMBER)

35. How many rooms are there in your house, not counting kitchens, bathrooms and toilets? (PLEASE FILL IN NUMBER)

36. At what age did the child's mother finish full-time education?
(PLEASE FILL IN AGE)

37. Which fuel is mainly used for cooking in your home?

Electricity	<input type="checkbox"/>
Gas	<input type="checkbox"/>
Other fuel	<input type="checkbox"/>

38. How do you heat your home? (PLEASE
TICK AS MANY AS APPLY)

Electric central heating	<input type="checkbox"/>
Gas central heating	<input type="checkbox"/>
Central heating with other fuel, e.g. oil	<input type="checkbox"/>
Heaters in rooms	<input type="checkbox"/>
Coal or wood fire	<input type="checkbox"/>

39. Is there visible damp within the house?

Yes	<input type="checkbox"/>
No	<input type="checkbox"/>

If there is visible damp, which rooms is it in?
(PLEASE TICK ALL THAT APPLY)

Kitchen	<input type="checkbox"/>
Bathroom	<input type="checkbox"/>
Child's bedroom	<input type="checkbox"/>
Other living areas	<input type="checkbox"/>

40. What type of flooring does your child have in his/her bedroom?

Carpet	<input type="checkbox"/>
Laminate	<input type="checkbox"/>
Laminate with rug	<input type="checkbox"/>
Other hard flooring	<input type="checkbox"/>
Other	<input type="checkbox"/>

41. Is your child exposed to animals?

(By exposed we mean do they come into close
contact with any animals on a regular basis)

Yes	<input type="checkbox"/>
No	<input type="checkbox"/>

If yes, which of the following animals?

(PLEASE TICK ALL THAT APPLY)

If your child is exposed to other animals that are not on the list,
please write which kind of animals in the space below:

.....

Cat	<input type="checkbox"/>
Dog	<input type="checkbox"/>
Bird	<input type="checkbox"/>
Fish	<input type="checkbox"/>
Rat	<input type="checkbox"/>
Gerbil	<input type="checkbox"/>
Rabbit	<input type="checkbox"/>
Hamster	<input type="checkbox"/>
Guinea pig	<input type="checkbox"/>
Sheep	<input type="checkbox"/>
Pigs	<input type="checkbox"/>
Cows	<input type="checkbox"/>
Horses	<input type="checkbox"/>

42. Does the child's mother smoke cigarettes?

Yes	<input type="checkbox"/>
No	<input type="checkbox"/>

If yes, how many per day?

1 to 10	<input type="checkbox"/>
11 to 20	<input type="checkbox"/>
More than 20	<input type="checkbox"/>

43. Do any other household members smoke cigarettes?

Yes	<input type="checkbox"/>
No	<input type="checkbox"/>

If yes, how many per day (total cigarettes smoked by
household members other than mother)?

1 to 10	<input type="checkbox"/>
11 to 20	<input type="checkbox"/>
More than 20	<input type="checkbox"/>

44. How would you best describe
the location of your house?

(PLEASE TICK THE ONE THAT
BEST APPLIES)

In a street with very dense traffic (main road)	<input type="checkbox"/>
In a street with moderate traffic (residential road)	<input type="checkbox"/>
In a quiet street with little or no traffic	<input type="checkbox"/>

45. Did you have any problems understanding this questionnaire?

Yes	<input type="checkbox"/>
No	<input type="checkbox"/>

Please write any comments you have about your child's health or about the questionnaire in the
space below:

.....
.....
.....
Thank you for completing the questionnaire. It will cost you nothing to return it if you use the pre-paid envelope provided.

For any queries please do not hesitate to contact us:

Ms Liz Lance, Dr Kaninika Basu; Contact: 01273 696955, ext 2404, 2353

Liz.Lance@bsuh.nhs.uk, k.basu@bsms.ac.uk

Filaggrin gene defects are associated with eczema, wheeze and nasal disease during infancy: prospective study

K Basu, MD^{1,2}, S K Inglis, PhD³, S A Bremner, PhD⁴, R Ramsay⁵, A Abd, MBChB, MSc², H Rabe, MD PhD^{2,5}, E Strange, BMBS², Veronica Phillips, PhD⁶, P Seddon, FRCPC^{2,5}, R Tavendale, PhD⁷, A Memon, DPhil⁴, C N A Palmer, PhD⁷, K Fidler, PhD^{2,5}, S Mukhopadhyay, PhD^{2,5}

¹ Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK; ² Academic Department of Paediatrics, Royal Alexandra Children's Hospital, Brighton and Sussex Medical School, Brighton, UK; ³ Tayside Clinical Trials Unit, University of Dundee, UK; ⁴ Department of Primary Care and Public Health, Brighton and Sussex Medical School, Brighton, UK; ⁵ Brighton and Sussex University Hospitals NHS Trust, Brighton, UK; ⁶ Medical Library, University of Cambridge, Cambridge, UK ⁷ Biomedical Research Institute, Ninewells Hospital and Medical School, University of Dundee, UK.

Corresponding author:

Dr Kaninika Basu, MD

Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK

Email: basukaninika@gmail.com

Declaration of financial interest: None

None of the authors disclose any conflict of interest statement.

Methods

The GO-CHILD study is a longitudinal study of a birth-cohort of 2312 infants born to mothers recruited from antenatal clinics in 8 different National Health Service (NHS) trusts in England and Scotland, between March 2009 and July 2015. The quality control procedures followed 'Good Clinical Practice' guidelines¹. The study is reported in accordance with the STROBE checklist.² The study was approved by the Tayside Committee on Medical Research and Ethics.

At all the sites, an information leaflet about the study was given to all expecting mothers at their 12-week scan appointment and at any other antenatal visit from 12 weeks gestation onwards. Informed written consent was obtained prior to recruitment. The children were followed-up until the age of 2 years. All neonates born at term were eligible for inclusion in the study; and infants with any perinatal insult such as perinatal asphyxia, significant respiratory difficulty, or congenital anomalies were excluded.

We estimated the required sample size based on the findings reported in a letter in Nature Genetics published a few years before we designed this study³. This study showed that a small sample size of the first publication and a large number of studies were independent predictors of discrepancies identified on subsequent meta-analyses of genetic association studies. The authors noted statistically significant discrepancies in 5 of 7 cases in which the first publications had a sample size of less than 150, compared with 3 of 29 when the sample size of the first study or studies was more than 150. We allowed for a relatively high level of attrition and aimed for an antenatal cohort size exceeding n=2000, aiming to achieve a sample size over 150 for all the analyses.

Detailed study design is described in online figure 1. The antenatal questionnaire collected information on family history of atopic conditions, environmental exposure to the child and parental smoking. The children were followed up for symptoms related to atopy, at the ages of 6, 12 and 24 months by postal questionnaires. These questionnaires were used to collect comprehensive information on respiratory, nasal and dermatological outcomes, precipitating environmental factors such as exposure to smoking and animals, and the effects of these conditions in relation to daily activities. These questions related to dry skin, eczema, wheeze, upper respiratory conditions and food allergies, and how these symptoms affected the child's life, including any visits to primary or secondary care and the prescribing of medication. For simplicity and greater accuracy through recall, only yes/no responses for any of the three options (yes, no, don't know) were used for analysis. The questionnaires were developed based on the Leicestershire questionnaire⁴ and were modified to make them relevant for younger children and also for the general population and not targeted towards children with asthma or wheeze.

'Wheeze' was defined as 'breathing that makes a high-pitched whistling or squeaking sound from the chest, not the throat' and 'rhinitis' was defined as 'a problem with sneezing, or a runny, or blocked nose when he/she did NOT have a cold or the flu'. 'Dry and itchy skin' was the parental report of generally dry and itchy skin but not including affected skin creases, 'respiratory impairment' was defined as any respiratory symptom affecting day-to-day life such as shortness of breath, disturbed sleep, dry nocturnal cough, and the nasal symptoms affecting day-to-day life comprised either or all of decreased daily activity due to rhinitis, snoring and sleep disturbance due to nasal symptoms.

Cord blood samples were collected at the time of delivery for genotyping. In absence of cord blood, a sample of saliva was collected in the postnatal period. Expecting mothers were invited through posters and leaflets to join the study at the time of their antenatal visits. Cord blood samples were collected at the time of delivery for genotyping. If this was unsuccessful, a sample of saliva (Oragene Neonatal Saliva Collection Kit, DNA Genotek, Ottawa, Canada K2G5W6) was collected in the postnatal period. The sample was collected by the researchers either at home or in the hospital, or posted to the researchers after collection at home by the family. The cord blood samples were stored at -80°C at the individual sites and later transported in batches to the Biomedical Research Institute, Dundee, for genotyping. The saliva was obtained from the infant using the Oragene Neonatal saliva sample collection kit (DNA Genotek, 29 Camelot Drive, Ottawa, Ontario, Canada K2G5W6). The sample was collected by the researchers either at home or in the hospital, or by the mother at home and posted to the researchers. All the genetic analyses are anonymised.

Genotyping for FLG R501X and 2282del4 was performed as described in our earlier publication⁵. AA refers to the wild- type FLG genotype for R501X, 2282del4, S3247X and R2447X mutations, Aa refers to heterozygous genotype for either of R501X, 2282del4, S3247X and R2447X, and aa refers to homozygous genotype for either of R501X, 2282del4, S3247X or R2447X. The homozygous, heterozygous and compound heterozygous genotypes were considered together as Aa/aa.

Data analyses were conducted using the IBM SPSS Statistics 146 software, Version 23 (IBM Corp., Armonk, and New York, USA), Stata version 15.1 (College Station, TX: StataCorp LLC) and Instat for Macintosh programmes. The chi-square test was used to compare the effects of the mutations on the atopic outcomes such as eczema, wheeze, rhinitis and dry or

itchy skin. Significance was assessed at $P < 0.05$. We have fitted log-binomial models to report relative risks and report estimates with 95% CIs; and we have additionally fitted log-binomial models by generalised estimating equations with an unstructured correlation matrix (to account for the lack of independence between repeated measurements, accepting that there are insufficient repeated measurements to estimate an autoregressive correlation structure). We report the results of the repeated measurements analysis in Table 5. We also performed binary logistic regression for the 4 atopic outcomes individually at each time point separately and subsequently log-binomial regressions for each outcome to obtain estimates of relative risk.

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126 McLean WH. Common loss of function variants of the epidermal barrier protein filaggrin are
127 a major predisposing factor for atopic dermatitis. *Nature Genetics* 2006; 38(4):441–446.

128

129

Time of contact

12 weeks gestation to delivery

Recruitment

Additional

Antenatal diet questionnaire

Collection of cord blood

Collection of saliva if cord blood

not obtained

3month dietary questionnaire

6month follow-up questionnaire

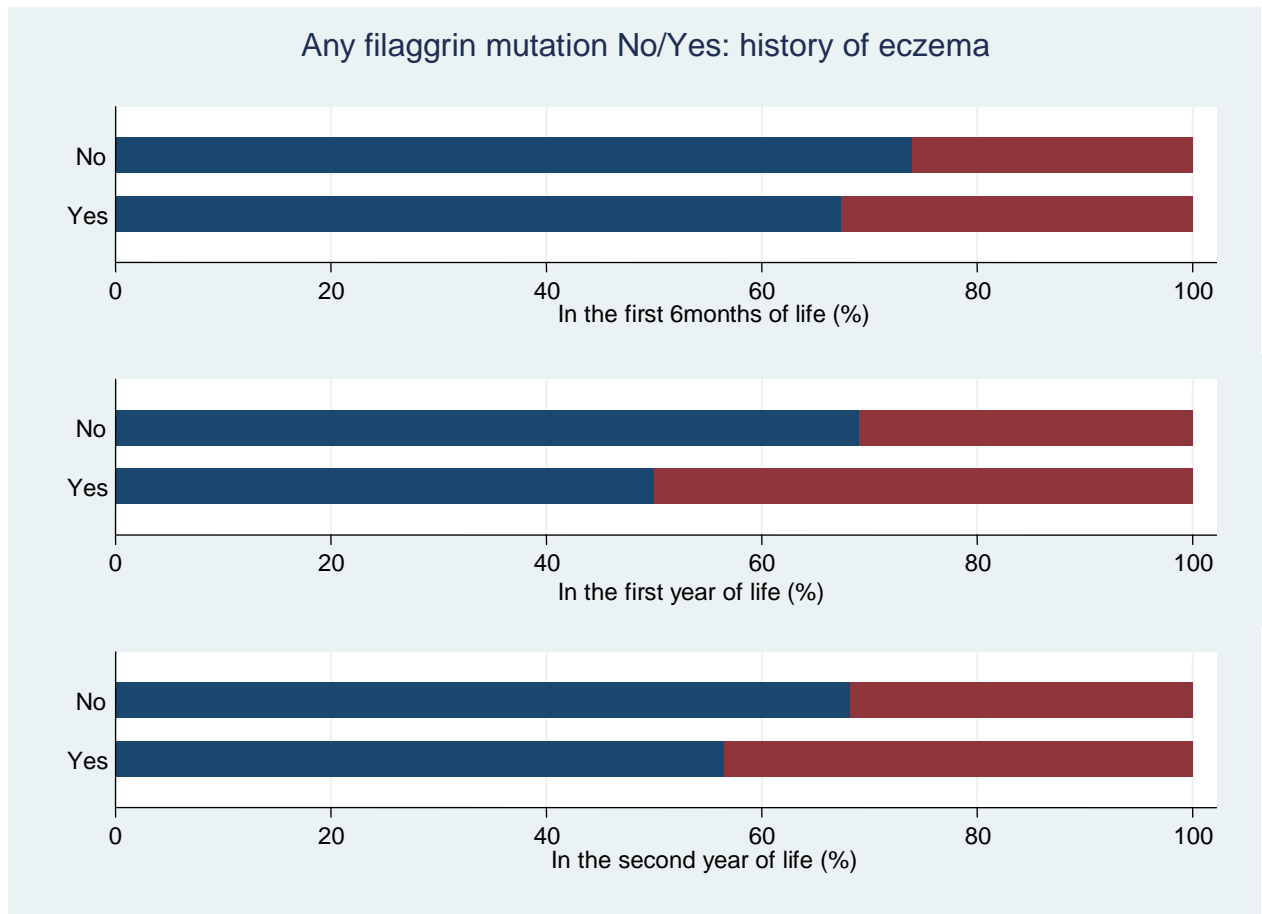
9month dietary questionnaire

12 month follow-up questionnaire

24 month follow-up questionnaire

Skin prick test

Figure 2: Incidence (%) of atopy-related clinical outcomes during the first 2 years of life in children where 6-month, 12-month and 24-month follow-up data are available (n=331)

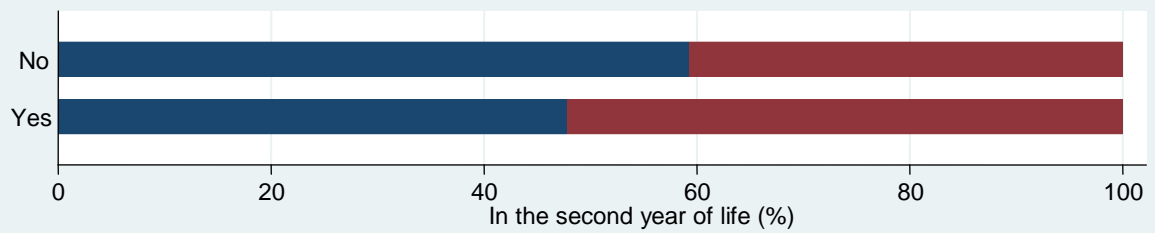
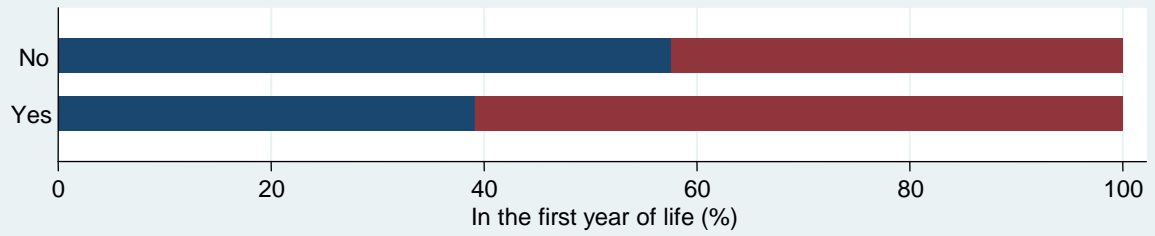
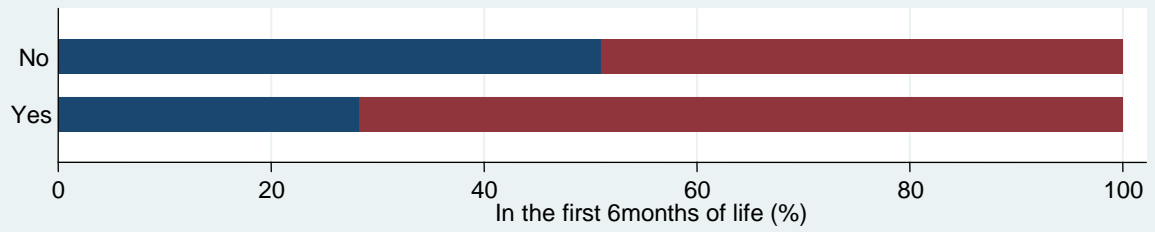


KEY:

Blue bar: No

Red bar: Yes

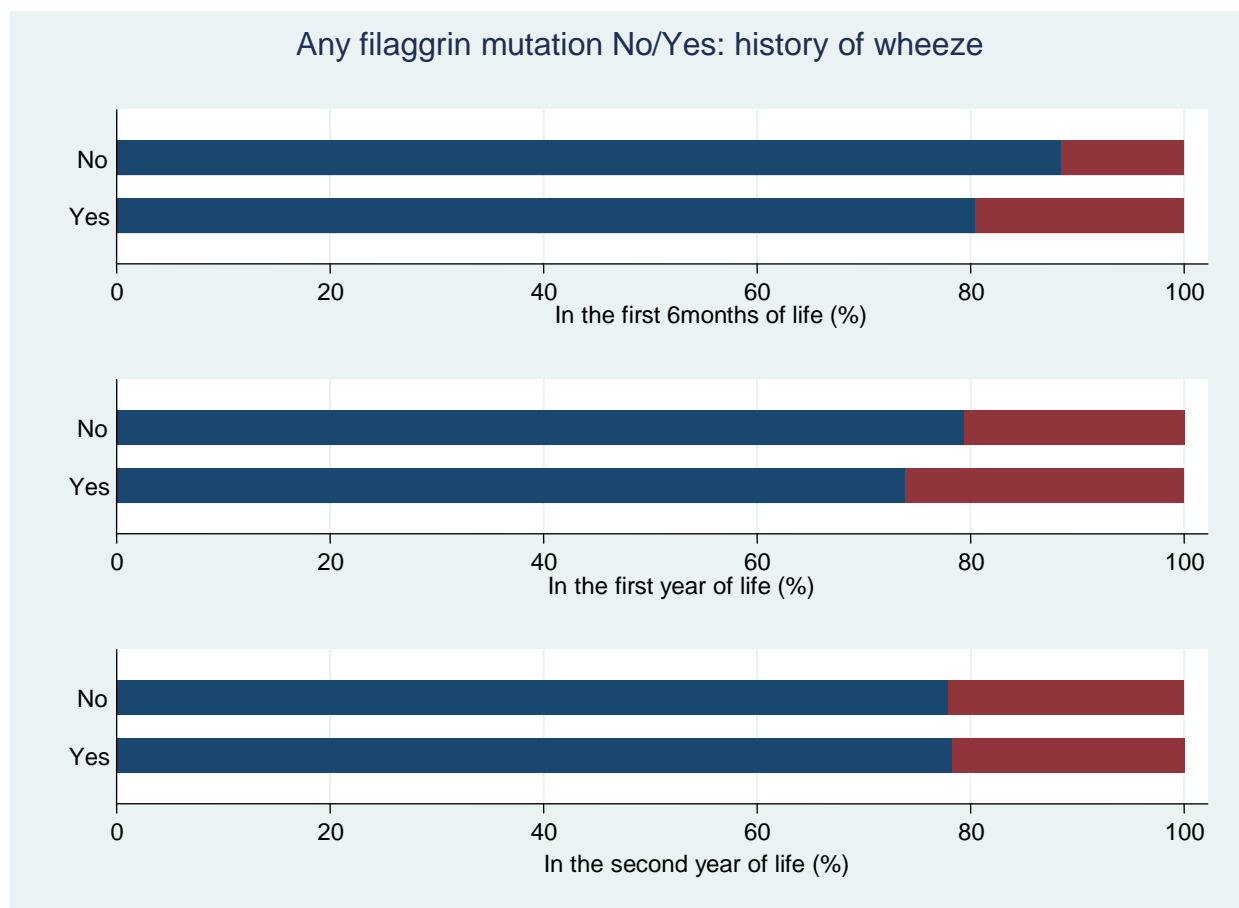
Any filaggrin mutation No/Yes: history of dry or itchy skin



KEY:

Blue bar: No

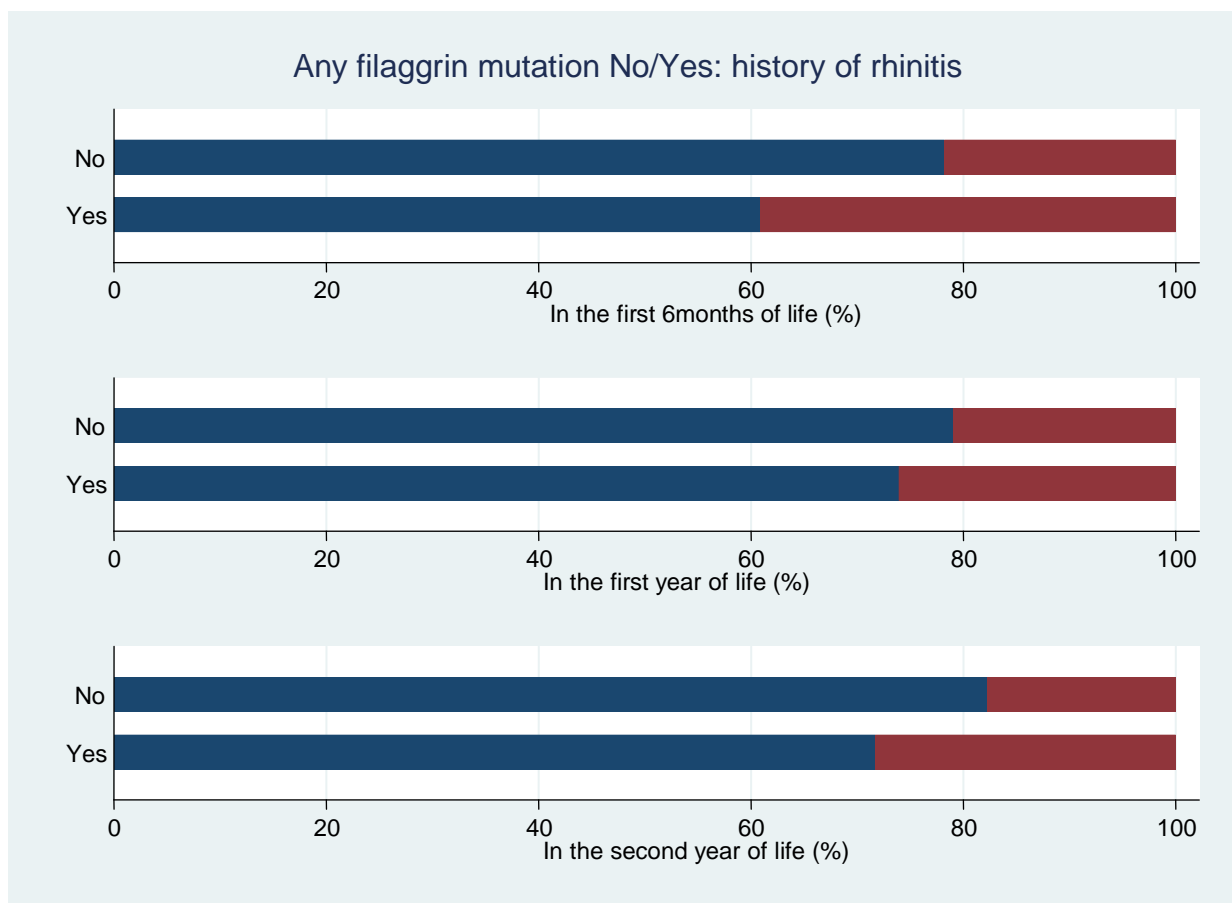
Red bar: Yes



KEY:

Blue bar: No

Red bar: Yes



KEY:

Blue bar: No

Red bar: Yes

**Filaggrin gene defects are associated with eczema, wheeze and nasal disease during infancy:
prospective study**

K Basu, MD^{1,2}, S K Inglis, PhD³, S A Bremner, PhD⁴, R Ramsay⁵, A Abd, MBChB, MSc², H Rabe, MD PhD^{2,5}, E Strange, BMBS², Veronica Phillips, PhD⁶, P Seddon, FRCPC^{2,5}, R Tavendale, PhD⁷, A Memon, DPhil⁴, C N A Palmer, PhD⁷, K Fidler, PhD^{2,5}, S Mukhopadhyay, PhD^{2,5}

¹ Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK; ² Academic Department of Paediatrics, Royal Alexandra Children's Hospital, Brighton and Sussex Medical School, Brighton, UK; ³ Tayside Clinical Trials Unit, University of Dundee, UK; ⁴ Department of Primary Care and Public Health, Brighton and Sussex Medical School, Brighton, UK; ⁵ Brighton and Sussex University Hospitals NHS Trust, Brighton, UK; ⁶ Medical Library, University of Cambridge, Cambridge, UK; ⁷ Biomedical Research Institute, Ninewells Hospital and Medical School, University of Dundee, UK.

Corresponding author:

Dr Kaninika Basu, MD

Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK

Email: basukaninika@gmail.com

Declaration of financial interest: None

None of the authors disclose any conflict of interest statement.

Table 1a. Characteristics of the GO-CHILD antenatal cohort (n=2312)

<i>FLG</i> mutation	AA: 1263 (88%) Aa/aa: 168 (12%)
Exposure to animals ¹	311 (13%)
Exposure to smoke	92 (4%)
Family history of asthma	1143 (49%)
Family history of eczema	975 (42%)
Family history of rhinitis	1134 (49%)
Ethnicity (n=1489)	Caucasian: 1387 (93%) (including mixed Caucasian 18) (1.2%) Asian: 26 (1.7%) Black: 16 (1.1%) Other: 11 (0.7%)

Table 1b. Number (%) of parent reported skin, respiratory and nasal symptoms

Clinical outcomes	During first 6 months (n=910*)	During first year (n=1176*)	During second year (n=962*)
Eczema	257 (28%)	372 (32%)	311 (32%)
Dry or itchy skin ¹	480 (53%)	559 (47%)	439 (46%)
Wheeze	123 (13%)	268 (23%)	206 (21%)
Respiratory impairment ²	96 (10%)	242 (21%)	188 (19%)
Rhinitis ³	236 (26%)	263 (22%)	195 (20%)
Nasal symptoms affecting day-to-day life ⁴	521 (57%)	545 (46%)	435 (45%)
Parental report of asthma	Not applicable	56 (5%)	62 (6%)

*Number of returned questionnaires

¹Dry or itchy skin is parental report of generally dry or itchy skin but not including affected skin creases

²Respiratory impairment is any respiratory symptom affecting day-to-day life such as shortness of breath, disturbed sleep, dry nocturnal cough

³ A problem with sneezing, or a runny, or blocked nose when he/she did not have a cold or the flu

⁴Nasal symptoms affecting day-to-day life is either or all of decreased daily activity due to rhinitis, snoring and sleep disturbance due to nasal symptoms

Table 2. Associations between *FLG* genotype (co-dominant and mutant variants) and eczema, dry or itchy skin, wheeze and rhinitis during the first 6 months of life (n=677*)

	Filaggrin	AA	Aa/aa	Total	Adjusted Relative Risk** (95% CI)
Eczema	No	448	44	492	1.82 (1.39, 2.39)
	Yes	150	35	185	
	Total	598	79	677	
Dry or itchy skin	No	304	22	326	†2.71 (1.61, 4.55)
	Yes	294	57	351	
	Total	598	79	677	
Wheeze	No	525	63	588	1.63 (1.00, 2.65)
	Yes	73	16	89	
	Total	598	79	677	
Rhinitis	No	449	50	499	1.46 (1.06, 2.01)
	Yes	149	29	178	
	Total	598	79	677	

KEY:

† Adjusted odds ratio as log-binomial model non-convergent

*Number of participants with *FLG* genotyping and returned questionnaires at 6 month time-point

** Adjusted for exposure to day care and animals

aa: Homozygous R501X or 2282del4 genotype or compound heterozygous genotype

Aa: Heterozygous genotype for either R501X or 2282del4

AA: Wild- type/ wild- type *FLG* genotype for R501X and 2282del4 mutation

Table 3. Associations between FLG genotype (co-dominant and mutant variants) and eczema, dry or itchy skin, wheeze and rhinitis during the first year of life (n=809*)

	Filaggrin	AA	Aa/aa	Total	Adjusted relative risk** (95% CI)
Eczema	No	507	46	553	1.80 (1.39, 2.32)
	Yes	207	49	256	
	Total	714	95	809	
Dry or itchy skin	No	399	30	429	†2.28 (1.32, 3.92)
	Yes	315	65	380	
	Total	714	95	809	
Wheeze	No	552	66	618	1.45 (0.98, 2.13)
	Yes	162	29	191	
	Total	714	95	809	
Rhinitis	No	560	70	630	1.48 (0.99, 2.22)
	Yes	154	25	179	
	Total	714	95	809	

KEY:

† Adjusted odds ratio as log-binomial model non-convergent

*Number of participants with *FLG* genotyping and returned questionnaires at 12 month time-point

** Adjusted for exposure to day care and animals

aa: Homozygous R501X or 2282del4 genotype or compound heterozygous genotype

Aa: Heterozygous genotype for either R501X or 2282del4

AA: Wild- type/ wild- type *FLG* genotype for R501X and 2282del4 mutation

Table 4. Associations between FLG genotype (co-dominant and mutant variants) and eczema, dry or itchy skin, wheeze and rhinitis during the second year of life (n=664*)

	Filaggrin	AA	Aa/aa	Total	Adjusted relative risk** (95% CI)
Eczema	No	409	46	455	1.40 (1.00, 1.97)
	Yes	171	38	209	
	Total	580	84	664	
Dry or itchy skin	No	332	32	364	†1.83 (1.02, 3.28)
	Yes	248	52	300	
	Total	580	84	664	
Wheeze	No	460	66	526	0.84 (0.47, 1.51)
	Yes	120	18	138	
	Total	580	84	664	
Rhinitis	No	468	60	528	1.38 (0.84, 2.25)
	Yes	112	24	136	
	Total	580	84	664	

KEY:

† Adjusted odds ratio as log-binomial model non-convergent

*Number of participants with *FLG* genotyping and returned questionnaires at 24 month time-point

** Adjusted for exposure to day care and animals

aa: Homozygous R501X or 2282del4 genotype or compound heterozygous genotype

Aa: Heterozygous genotype for either R501X or 2282del4

AA: Wild- type/ wild- type *FLG* genotype for R501X and 2282del4 mutation

Table 5. Associations between FLG genotype (co-dominant and mutant variants) and eczema, dry and itchy skin, wheeze and rhinitis across 6, 12 and 24 months of life (n=677)

Outcome	Adjusted relative risk**	95% CI
Eczema	1.70	(1.38, 2.11)
Dry or itchy skin (n=559)	†2.02	(1.25, 3.26)
Wheeze	1.29	(0.92, 1.81)
Rhinitis	1.42	(1.11, 1.83)

† Adjusted odds ratio as log-binomial model non-convergent

** adjusted for exposure to day care and animals

aa: Homozygous R501X or 2282del4 genotype or compound heterozygous genotype

Aa: Heterozygous genotype for either R501X or 2282del4

AA: Wild- type/ wild- type *FLG* genotype for R501X and 2282del4 mutation